

# PATENT ABSTRACTS OF JAPAN

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(54) PRODUCTION OF OIL AND FAT CONTAINING TRIGLYCERIDE CONTAINING GAMMA-LINOLENIC ACID AT HIGH CONCENTRATION AND/OR TRIGLYCERIDE CONTAINING DIHOMO-GAMMA-LINOLENIC ACID AT HIGH CONCENTRATION

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a method for production by which a triglyceride containing  $\gamma$ -linolenic acid or dihomo- $\gamma$ -linolenic acid excellent in preservation stability at a high concentration is continuously obtained for a long period.

SOLUTION: A lipase acting only on ester bonds at the 1,3-positions of triglycerides is reacted with an oil and fat containing a  $\gamma$ -linolenic acid- containing triglyceride and/or a dihomo- $\gamma$ -linolenic acid-containing triglyceride in the presence of a medium-chain fatty acid and 30-500 ppm water.

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## CLAIMS

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### [Claim(s)]

[Claim 1]In included fats and oils, gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride under medium chain fatty acid and 30-500 ppm existence of water, A manufacturing method of fats and oils containing gamma-linolenic acid advanced content triglyceride and/or dihome-gamma-linolenic acid advanced content triglyceride making lipase which acts only on an ester bond like 1,3- of triglyceride react.

[Claim 2]A manufacturing method of fats and oils containing the gamma-linolenic acid advanced content triglyceride according to claim 1 and/or dihome-gamma-linolenic acid advanced content triglyceride making it react under existence of vitamin E. [Claim 3]A manufacturing method of fats and oils containing the gamma-linolenic acid advanced content triglyceride according to claim 1 or 2 and/or dihome-gamma-linolenic acid advanced content triglyceride using fixed lipase as lipase.

[Claim 4]Fats and oils containing gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride, a claim extracting from vegetation, algae, or fungi -- a manufacturing method of fats and oils which contain gamma-linolenic acid advanced content triglyceride of a statement, and/or dihome-gamma-linolenic acid advanced content triglyceride one to 3 either.

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## DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the manufacturing method of gamma-linolenic acid advanced content triglyceride and/or dihome-gamma-linolenic acid advanced content triglyceride.

[0002]

[Description of the Prior Art] In recent years, the physiology activity which higher unsaturated fatty acid content TORIGU ceride has attracts attention. It is known that especially gamma-linolenic acid content triglyceride and dihome-gamma-linolenic acid content triglyceride have many bioactive operations, such as an improving action, a carcinostatic operation, an immunostimulatory action to adult diseases, such as atopic dermatitis, arthritis-chronica rheumatism, and hypertension. And various examination about the directions to the drugs of gamma-linolenic acid content triglyceride or dihome-gamma-linolenic acid content triglyceride and a food for specified health use is made.

[0003] Conventionally, the loss of a higher unsaturated fatty acid is lessened, and the method of manufacturing the triglyceride which contains a higher unsaturated fatty acid in high concentration is demanded, without carrying out the byproduction of the diglyceride. For example, in JP,63-273485,A. polyunsaturated-fatty-acid content fats and oils and saturated fatty acid -- and -- or saturated fatty acid alcohol ester <sup>using</sup> specific lipase, Carry out an ester interchange and the triglyceride which has polyunsaturated-fatty-acid acid at least in 2- is contained 40% of the weight or more, And the process of the oil and fat composition in which all the with a carbon numbers of 16 or more saturated fatty acid content is 50 % of the weight or more is indicated, and in JP,6-287594,A. The manufacturing method of the triglyceride which uses fish oil and oleic acid as a raw material, contains oleic acid at least in 1,3- using the ester exchange reaction using specific lipase, and contains docosahexaenoic acid at least in 2- is indicated. In JP,8-214891,A, the manufacturing method of the fats and oils on which the lipase which acts only on 1 of triglyceride and the ester bond like 3-is made to act under existence of fats and oils and medium chain fatty acid is indicated.

[0004]

[Problem(s) to be Solved by the Invention] However, in a method given in JP,63-273485,A. There is a problem that the specific higher unsaturated fatty acid in triglyceride cannot be condensed highly, and in a method given in JP,6-287594,A. Since the oleic acid used for an ester interchange was equivalent to the average molecular weight of the constituent fatty acids of fish oil, the gamma-linolenic acid content in

triglyceride could not be raised, and the yield of triglyceride in formed oil fat was not so high as about 90-mol % to raw material triglyceride. In a method given in JP,8-214891,A, Although the moisture content (0 to 1000%) to the amount of enzymes is mentioned, in the example, as much as 2.5g (12,300 ppm) per 202.5g of systems of reaction of moisture is used, as a result of this invention persons' examining a moisture content, even if it applied this moisture content to gamma-linolenic acid content triglyceride, it became clear that the concentration of the higher unsaturated fatty acid in triglyceride was low, and what is still satisfied does not have profit.

[0005]

[Means for Solving the Problem]In then, fats and oils which contain gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride as a result of this invention persons' inquiring wholeheartedly in light of the above-mentioned circumstances. By making lipase which acts only on 1 of triglyceride, and an ester bond like 3-react under medium chain fatty acid and 30-500 ppm existence of water. Medium chain fatty acid whose molecular weight is smaller than higher unsaturated fatty acids, such as gamma-linolenic acid or dihome-gamma-linolenic acid, is introduced at least into 1 and 3-, By being held as it is, gamma-linolenic acid or dihome-gamma-linolenic acid which exists at least in 2-, triglyceride (below gamma-linolenic acid advanced-content triglyceride.) in which a content of gamma-linolenic acid and dihome-gamma-linolenic acid became large as a result dihome-gamma-linolenic acid advanced content triglyceride -- calling -- yield is good, it succeeds in manufacturing continuously for a long period of time, the preservation stability of fats and oils containing triglyceride obtained further finds out a good thing, and it came to complete this invention.

[0006]Hereafter, this invention is explained in detail. Although fats and oils containing gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride (it is written as raw material triglyceride below) are used in this invention, These fats and oils are what contains triglyceride containing gamma-linolenic acid or dihome-gamma-linolenic acid in constituent fatty acids, For example, fats and oils further extracted from algae, such as chlorella and Spirulina, and fungi of Mortierella besides vegetation, such as oleum rapae, Oenotherae Biennis oil, a black gooseberry oil, and a BORAJI oil, can be mentioned.

[0007]As medium chain fatty acid of this invention, it is a carbon number. Although it is chosen out of fatty acid which has 6-12 pieces and caproic acid, caprylic acid, capric acid, lauryl acid, etc. are mentioned, caprylic acid and capric acid are used preferably.

[0008]As lipase used by this invention, what microorganisms, such as the Rhizopus (Rhizopus) group, a RIZOMU call (Rhizomucor) group, and an Aspergillus (Aspergillus) group, produce, a swine pancreatic lipase, etc. are mentioned, for example. A commercial thing can be used about this lipase. for example, lipase (the Tanabe Seiyaku Co., Ltd. make.) of Rhizopus delemar (Rhizopus delemar) lipase (a product made by Novo NORUTISUKU.) of "TARIPAZE" RIZOMU call MIIHEI (Rhizomucor miehei) Lipase (Amano Pharmaceuticals, "lipase A") of "ribozyme IM" and Aspergillus-niger (Aspergillus niger), etc. are mentioned.

[0009]In this invention, since a moisture content can be set to 0 if fixed lipase is used as this lipase, it is effective in respect of adjustment of a moisture content of the system of reaction mentioned later.

[0010]As a carrier to fix, although cerite, ion-exchange resin, ceramics, etc. are mentioned, ceramics are used preferably, and although ceramic carrier SM-10 (made by NGK Insulators, Ltd.) is preferred as a kind of ceramics, it is not limited to this. When using fixed lipase, the amount of lipase is 1,000 to 300,000 unit preferably 100 to 2,000,000 unit per 1g of carriers.

[0011]0.1 to 30 % of the weight which contains lipase of the above-mentioned number of units especially as a fixing method of lipase, for example although not limited, Making 1-ag [ 10 ] ceramic carrier preferably suspended to 10-30 ml, and 1.0 to 20% of the weight of protein (lipase) solution [ 1-100 ml of ] agitating loosely preferably. -Add gradually 10-300 ml of acetone, ethanol, or isopropanol cooled at 20-80 \*\*, and make lipase stick to immobilization support. Fractions which precipitated are collected and it dries enough under decompression conditions.

[0012]Next, fixed lipase is activated. In the case of a batch method, activation is the fats and oils / medium chain fatty acid / water of ten to 50 time capacity of immobilized enzyme. [30-40:60-70:1-5 (weight ratio)] 30 \*\* incubates in inside, making it shake for 40 hours. In the case of a column method, activation is the fats and oils / medium chain fatty acid / water 1-10-times the amount of immobilized enzyme. [30-40:60-70:5-10 (weight ratio)] Mixed liquor is dipped and passage liquid is again added in a column. It is the sum total about this operation, and is 1 - 10 cycle \*\*\*\*. The rate of flow in this case is good without limit, if there is no pressure loss.

[0013]Although the ester interchange of the higher unsaturated fatty acid like 1,3-in raw material triglyceride is carried out to medium chain fatty acid with lipase in a manufacturing method of this invention, as a quantity of fats and oils containing raw material triglyceride in the system of reaction, it is 15 to 50 % of the weight preferably ten to 50% of the weight. As a quantity of medium chain fatty acid in the system of reaction, 50 to 90% of the weight, it is 50 to 85 % of the weight preferably, and as for a weight ratio of the raw material triglyceride / medium chain fatty acid in the system of reaction, 1-10 are preferred, and also they are 1-5.

[0014]An addition in the system of reaction of lipase has four to 80,000 preferred unit to 1 g of reaction mixture, and also is 40 to 8,000 unit. One unit here uses olive oil as a substrate, and shows the amount of lipase required to generate fatty acid of a 1micro mol in 1 minute.

[0015]In this invention, it is characterized [ greatest ] by making it react under 30-500 ppm existence of water in the case of this reaction, and is 50-150 ppm preferably. If an ester interchange becomes difficult to advance in less than 30 ppm and water exceeds 500 ppm, since the stability of an enzyme will worsen and hydrolysis of triglyceride will take place, it is not desirable. Although water is contained in fats and oils containing lipase, medium chain fatty acid, and raw material triglyceride, quantity of total water, It is required to control to be set to 30-500 ppm, and as the method of this control, \*\* Although a moisture content of each ingredient is measured with a Karl Fischer technique and there are a method of controlling a total moisture content, a method of drying \*\* reaction component thoroughly and adding water of the specified quantity later, etc. beforehand, since handling of some hygroscopic things, such as powdered lipase, is simple, a method of \*\* is preferred. A moisture content which fixed lipase holds shall not be included in a moisture content of this invention.

[0016]Although both a batch method and a column method are applicable as a reaction method, since the possibility of a reaction and solid liquid separation are continuously easy for a large quantity, a column method is preferred.

[0017]Although a column method which uses fixed lipase below is explained, it is not limited to this. First, a column is filled up with immobilized enzyme and they are the fats and oils / medium chain fatty acid / water of one to 10 time capacity of immobilized enzyme. [30-40:60-70:5-10 (weight ratio)] As a place of activation of the above-mentioned fixed lipase described with mixed liquor, a total of four cycles are dipped and it is

activated. Subsequently, if each moisture content of fats and oils and medium chain fatty acid containing raw material triglyceride dried by vacuum distillation, dehydrating treatment, etc. is measured and there is necessity, as the specified quantity addition of the water is carried out and it becomes a moisture content of 30-500 ppm, it will agitate uniformly, and a reaction stock solution will be produced. this reaction stock solution -- linear velocity 0.5 - 1000 ml/hr -- desirable -- 1 - 10 ml/hr, and the space velocity 0.01-10/hr -- a column is preferably passed by 1 [ 0.1-] /hr. Reaction temperature of 10-60 \*\* is 15-45 \*\* preferably.

[0018]After neutralizing superfluous medium chain fatty acid which was made to add and carry out the ester interchange of the alkali to obtained passage liquid, and was produced and which was not exchanged for free fatty acid and considering it as fatty acid salt, water is added, this fatty acid salt is extracted to a water layer, an organic solvent is added, and triglyceride (oil reservoir) is collected. A water layer can also be recycled and used for the system of reaction.

[0019]by the above-mentioned ester synthetic reaction, triglyceride receives raw material triglyceride -- 93-97-mol % -- it can collect and dihome-gamma-linolenic acid can be made into 25 to 36 % of the weight for gamma-linolenic acid in triglyceride to 28 to 35% of the weight.

[0020]In this invention, in a reaction of the above-mentioned lipase, it is preferred to also make vitamin E live together, and it contributes to improvement in the preservation stability of fats and oils containing manufactured triglyceride, handling nature, etc. As this vitamin E, either or mixtures, such as alpha-tocopherol, beta-tocopherol, gamma-tocopherol, and delta-tocopherol, are used, and wheat \*\*\*\*\* etc. are mentioned preferably.

[0021]In order that triglyceride manufactured by this invention may not separate at all gamma-linolenic acid or dihome-gamma-linolenic acid mostly contained at least in 2-, Contain highly gamma-linolenic acid or dihome-gamma-linolenic acid, and in a manufacturing method of this invention. When an ester exchange reaction by the above-mentioned column method is performed continuously, a reaction which obtains triglyceride with a recovery rate beyond 95 mol % to raw material triglyceride can be run continuously about 30 to 200 days. Even if it allows fats and oils containing obtained triglyceride to stand at a room temperature for a long period of time, there are few rises of acid value, and preservation stability is good.

[0022]

[Example]Hereafter, an example explains this invention still more concretely. However, this invention is not limited to these examples. That it is with "%" shows peak area % which analyzed fatty acid composition by the gas chromatograph.

Example 1 ceramic-carrier SM-10 the [NGK Insulators, Ltd. make] -- Rhizopus Delmer's (Rhizopus delemar) lipase (the Tanabe Seiyaku Co., Ltd. make.) From the upper bed of a column to the BORAJI oil after fixing "TARIPAZE" 5,000 unit / carrier g8g and stuffing the column (1.5-cm [ in diameter ], 6.2-cm [ in length ], and capacity 10.95cm<sup>3</sup>) of a cylindrical shape It is wheat germ oil (Eisai Co., Ltd. make, "IMIKKUSU") 0.2 % of the weight] as 22.2% of gamma-linolenic acid in triglyceride, [moisture content content of 200 ppm, and vitamin E. 1:2 (weight ratio) mixture of the caprylic acid (moisture content content of 200 ppm) which is medium chain fatty acid of the carbon number 8 [raw-material triglyceride: The ester interchange successive reaction was performed at 30 \*\*, teaching caprylic acid =1:2(weight ratio)] by linear velocity 4 ml/hr, and space velocity 0.589 / hr. Passage liquid 3g preparative isolation of the obtained reaction mixture was done the one-day back of a reaction start, and 90 days afterward, it added 1N-sodium hydroxide solution, neutralized, removed the water layer (lower layer) after neglect, carried out hexane extraction of the

triglyceride layer (upper layer), removed this hexane, and obtained the glyceride fraction. The ODS column (AM120 S-50, product made by YMC) analyzed this glyceride fraction, and the triglyceride fraction was computed with 0.79 g. Methyl esterification of the obtained triglyceride fraction was carried out with the conventional method, capillary chromatography analyzed the fatty acid composition in triglyceride, and gamma-linolenic acid became 29.4%. [Since the triglyceride 0.79g (29.4% of gamma-linolenic acid) was obtained from 1g of raw material triglyceride (22.2% of gamma-linolenic acid), the recovery rate of triglyceride was 96-mol %.] These fats and oils 1g were put into the seal test tube, the retention test was done for one month at the room temperature, the acid value before preservation and after preservation was measured by the standard fats-and-oils assay method, the rise of acid value was measured, and it evaluated as follows.

O ... [ ... As for more than 10mgKOH / fats and oils g, the result one day after a reaction start is shown in Table 1, and the result of 90 days after is shown in Table 2. ] 1mgKOH / less than fats-and-oils g O ... 1 - 5mgKOH / less than fats-and-oils g \*\* ... 5 - 10mgKOH / less than fats-and-oils g x

[0023]In example 2 Example 1, except having used the BORAJI oil which does not contain a wheat germ oil (the Eisai Co., Ltd. make, "IMIKKUSU"), it reacted similarly, and analyzed similarly and content triglyceride was obtained for gamma-linolenic acid at recovery rate % of 94 mol to the raw material BORAJI oil 29.0%. The retention test was done similarly. A result is shown in Tables 1 and 2.

[0024]In example 3 Example 1, instead of caprylic acid, capric acid (moisture content of 200 ppm) was carried out to \*\*\*\* for the said weight, and the appearance, and was evaluated similarly. A result is shown in Tables 1 and 2.

[0025]In example 4 Example 1, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0026]In example 5 Example 2, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0027]In example 6 Example 3, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0028]In comparative example 1 Example 1, used a 10 ppm thing for 10 ppm, the moisture content of caprylic acid was made to react to them like Example 1, the moisture content of the BORAJI oil was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0029]In comparative example 2 Example 1, used a 1000 ppm thing for 1000 ppm, the moisture content of caprylic acid was made to react to them like Example 1, the moisture content of the BORAJI oil was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0030]

[Table 1]

	Triglyceride Retention test	GLA(%)	DGLA(%)	TORIGURISEDO raw material	After a reaction	Raw material	After a reaction
Recovery rate (mol %)	example 1	22.2	29.4	---	---	96	O
example 2	22.2	29.0	---	---	94	O	
example 3	22.2	29.0	---	---	96	O	
example 4	---	16.0	25.3	95	O		
example 5	---	16.0	25.0	95	O		
example 6	---	16.025	0	90	O		
comparative example 1	22.0	28.5	---	98	O		
comparative example 2	22.0	28.0	---	90	O		

[0031]

[Table 2]

Triglyceride Retention test GLA(%) DGLA(%) TORIGURISEDO raw material After a reaction Raw material After a reaction Recovery rate (mol %) example 1 22.2 29.2 -- --- 95 O Example 2 22.2 29.0 -- --- 94 O Example 3 22.2 29.0. -- --- 96 O Example 4 -- -- 16.0 25.3 95 O Example 5 --- 16.0 25.0 95 O example 6 --- 16.0 25.0 95 O comparative example 1 22.0 22.2 -- --- 100 O comparative example 2 22.0 23.0 -- --- 75 O[0032]

[Effect of the Invention]In this invention, gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride in the included fats and oils under medium chain fatty acid and 30-500 ppm existence of water, Since the lipase which acts only on the ester bond like 1,3-of triglyceride is made to react, yield is good and the preservation stability of the fats and oils containing the triglyceride which it became possible for this triglyceride to be obtained and for it to be made to react continuously in a column for a long period of time using fixed lipase, and was obtained further is good.

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**TECHNICAL FIELD**

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[Field of the Invention] This invention relates to the manufacturing method of gamma-linolenic acid advanced content triglyceride and/or dihome-gamma-linolenic acid advanced content triglyceride.

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## PRIOR ART

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[Description of the Prior Art] In recent years, the physiology activity which higher unsaturated fatty acid content TORIGU ceride has attracts attention. It is known that especially gamma-linolenic acid content triglyceride and dihome-gamma-linolenic acid content triglyceride have many bioactive operations, such as an improving action, a carcinostatic operation, an immunostimulatory action to adult diseases, such as atopic dermatitis, arthritis-chronica rheumatism, and hypertension. And various examination about the directions to the drugs of gamma-linolenic acid content triglyceride or dihome-gamma-linolenic acid content triglyceride and a food for specified health use is made.

[0003] Conventionally, the loss of a higher unsaturated fatty acid is lessened, and the method of manufacturing the triglyceride which contains a higher unsaturated fatty acid in high concentration is demanded, without carrying out the byproduction of the diglyceride. For example, in JP,63-273485,A. polyunsaturated-fatty-acid content fats and oils and saturated fatty acid -- and -- or saturated fatty acid alcohol ester <sup>using</sup> specific lipase, Carry out an ester interchange and the triglyceride which has polyunsaturated-fatty-acid acid at least in 2- is contained 40% of the weight or more, And the process of the oil and fat composition in which all the with a carbon numbers of 16 or more saturated fatty acid content is 50 % of the weight or more is indicated, and in JP,6-287594,A. The manufacturing method of the triglyceride which uses fish oil and oleic acid as a raw material, contains oleic acid at least in 1,3- using the ester exchange reaction using specific lipase, and contains docosahexaenoic acid at least in 2- is indicated. In JP,8-214891,A, the manufacturing method of the fats and oils on which the lipase which acts only on 1 of triglyceride and the ester bond like 3-is made to act under existence of fats and oils and medium chain fatty acid is indicated.

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#### EFFECT OF THE INVENTION

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[Effect of the Invention]In this invention, gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride in the included fats and oils under medium chain fatty acid and 30-500 ppm existence of water, Since the lipase which acts only on the ester bond like 1,3-of triglyceride is made to react, yield is good and the preservation stability of the fats and oils containing the triglyceride which it became possible for this triglyceride to be obtained and for it to be made to react continuously in a column for a long period of time using fixed lipase, and was obtained further is good.

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## TECHNICAL PROBLEM

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[Problem(s) to be Solved by the Invention] However, in a method given in JP,63-273485,A. There is a problem that the specific higher unsaturated fatty acid in triglyceride cannot be condensed highly, and in a method given in JP,6-287594,A. Since the oleic acid used for an ester interchange was equivalent to the average molecular weight of the constituent fatty acids of fish oil, the gamma-linolenic acid content in triglyceride could not be raised, and the yield of triglyceride in formed oil fat was not so high as about 90-mol % to raw material triglyceride. In a method given in JP,8-214891,A, Although the moisture content (0 to 1000%) to the amount of enzymes is mentioned, in the example, as much as 2.5g (12,300 ppm) per 202.5g of systems of reaction of moisture is used, as a result of this invention persons' examining a moisture content, even if it applied this moisture content to gamma-linolenic acid content triglyceride, it became clear that the concentration of the higher unsaturated fatty acid in triglyceride was *low*, and what is still satisfied does not have profit.

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## MEANS

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[Means for Solving the Problem] In then, fats and oils which contain gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride as a result of this invention persons' inquiring wholeheartedly in light of the above-mentioned circumstances. By making lipase which acts only on 1 of triglyceride, and an ester bond like 3-react under medium chain fatty acid and 30-500 ppm existence of water. Medium chain fatty acid whose molecular weight is smaller than higher unsaturated fatty acids, such as gamma-linolenic acid or dihome-gamma-linolenic acid, is introduced at least into 1 and 3-, By being held as it is, gamma-linolenic acid or dihome-gamma-linolenic acid which exists at least in 2-, triglyceride (below gamma-linolenic acid advanced-content triglyceride.) in which a content of gamma-linolenic acid and dihome-gamma-linolenic acid became large as a result dihome-gamma-linolenic acid advanced content triglyceride -- calling -- yield is good, it succeeds in manufacturing continuously for a long period of time, the preservation stability of fats and oils containing triglyceride obtained further finds out a good thing, and it came to complete this invention.

[0006] Hereafter, this invention is explained in detail. Although fats and oils containing gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride (it is written as raw material triglyceride below) are used in this invention, These fats and oils are what contains triglyceride containing gamma-linolenic acid or dihome-gamma-linolenic acid in constituent fatty acids, For example, fats and oils further extracted from algae, such as chlorella and Spirulina, and fungi of Mortierella besides vegetation, such as oleum rapae, Oenotherae Biennis oil, a black gooseberry oil, and a BORAJI oil, can be mentioned.

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used preferably, and although ceramic carrier SM-10 (made by NGK Insulators, Ltd.) is preferred as a kind of ceramics, it is not limited to this. When using fixed lipase, the amount of lipase is 1,000 to 300,000 unit preferably 100 to 2,000,000 unit per 1g of carriers.

[0011]0.1 to 30 % of the weight which contains lipase of the above-mentioned number of units especially as a fixing method of lipase, for example although not limited, Making 1-ag [ 10 ] ceramic carrier preferably suspended to 10-30 ml, and 1.0 to 20% of the weight of protein (lipase) solution [ 1-100 ml of ] agitating loosely preferably. -Add gradually 10-300 ml of acetone, ethanol, or isopropanol cooled at 20-80 \*\*, and make lipase stick to immobilization support. Fractions which precipitated are collected and it dries enough under decompression conditions.

[0012]Next, fixed lipase is activated. In the case of a batch method, activation is the fats and oils / medium chain fatty acid / water of ten to 50 time capacity of immobilized enzyme. [30-40:60-70:1-5 (weight ratio)] 30 \*\* incubates in inside, making it shake for 40 hours. In the case of a column method, activation is the fats and oils / medium chain fatty acid / water 1-10-times the amount of immobilized enzyme. [30-40:60-70:5-10 (weight ratio)] Mixed liquor is dipped and passage liquid is again added in a column. It is the sum total about this operation, and is 1 - 10 cycle \*\*\*\*. The rate of flow in this case is good without limit, if there is no pressure loss.

[0013]Although the ester interchange of the higher unsaturated fatty acid like 1,3-in raw material triglyceride is carried out to medium chain fatty acid with lipase in a manufacturing method of this invention, as a quantity of fats and oils containing raw material triglyceride in the system of reaction, it is 15 to 50 % of the weight preferably ten to 50% of the weight. As a quantity of medium chain fatty acid in the system of reaction, 50 to 90% of the weight, it is 50 to 85 % of the weight preferably, and as for a weight ratio of the raw material triglyceride / medium chain fatty acid in the system of reaction, 1-10 are preferred, and also they are 1-5.

[0014]An addition in the system of reaction of lipase has four to 80,000 preferred unit to 1 g of reaction mixture, and also is 40 to 8,000 unit. One unit here uses olive oil as a substrate, and shows the amount of lipase required to generate fatty acid of a 1micro mol in 1 minute.

[0015]In this invention, it is characterized [ greatest ] by making it react under 30-500 ppm existence of water in the case of this reaction, and is 50-150 ppm preferably. If an ester interchange becomes difficult to advance in less than 30 ppm and water exceeds 500 ppm, since the stability of an enzyme will worsen and hydrolysis of triglyceride will take place, it is not desirable. Although water is contained in fats and oils containing lipase, medium chain fatty acid, and raw material triglyceride, quantity of total water, It is required to control to be set to 30-500 ppm, and as the method of this control, \*\* Although a moisture content of each ingredient is measured with a Karl Fischer technique and there are a method of controlling a total moisture content, a method of drying \*\* reaction component thoroughly and adding water of the specified quantity later, etc. beforehand, since handling of some hygroscopic things, such as powdered lipase, is simple, a method of \*\* is preferred. A moisture content which fixed lipase holds shall not be included in a moisture content of this invention.

[0016]Although both a batch method and a column method are applicable as a reaction method, since the possibility of a reaction and solid liquid separation are continuously easy for a large quantity, a column method is preferred.

[0017]Although a column method which uses fixed lipase below is explained, it is not limited to this. First, a

column is filled up with immobilized enzyme and they are the fats and oils / medium chain fatty acid / water of one to 10 time capacity of immobilized enzyme. [30-40:60-70:5-10 (weight ratio)] As a place of activation of the above-mentioned fixed lipase described with mixed liquor, a total of four cycles are dipped and it is activated. Subsequently, if each moisture content of fats and oils and medium chain fatty acid containing raw material triglyceride dried by vacuum distillation, dehydrating treatment, etc. is measured and there is necessity, as the specified quantity addition of the water is carried out and it becomes a moisture content of 30-500 ppm, it will agitate uniformly, and a reaction stock solution will be produced. this reaction stock solution -- linear velocity 0.5 - 1000 ml/hr -- desirable -- 1 - 10 ml/hr, and the space velocity 0.01-10/hr -- a column is preferably passed by 1 [ 0.1-]/hr. Reaction temperature of 10-60 \*\* is 15-45 \*\* preferably.

[0018]After neutralizing superfluous medium chain fatty acid which was made to add and carry out the ester interchange of the alkali to obtained passage liquid, and was produced and which was not exchanged for free fatty acid and considering it as fatty acid salt, water is added, this fatty acid salt is extracted to a water layer, an organic solvent is added, and triglyceride (oil reservoir) is collected. A water layer can also be recycled and used for the system of reaction.

[0019]by the above-mentioned ester synthetic reaction, triglyceride receives raw material triglyceride -- 93-97-mol % -- it can collect and dihome-gamma-linolenic acid can be made into 25 to 36 % of the weight for gamma-linolenic acid in triglyceride to 28 to 35% of the weight.

[0020]In this invention, in a reaction of the above-mentioned lipase, it is preferred to also make vitamin E live together, and it contributes to improvement in the preservation stability of fats and oils containing manufactured triglyceride, handling nature, etc. As this vitamin E, either or mixtures, such as alpha-tocopherol, beta-tocopherol, gamma-tocopherol, and delta-tocopherol, are used, and wheat \*\*\*\*\* etc. are mentioned preferably.

[0021]In order that triglyceride manufactured by this invention may not separate at all gamma-linolenic acid or dihome-gamma-linolenic acid mostly contained at least in 2-, Contain highly gamma-linolenic acid or dihome-gamma-linolenic acid, and in a manufacturing method of this invention. When an ester exchange reaction by the above-mentioned column method is performed continuously, a reaction which obtains triglyceride with a recovery rate beyond 95 mol % to raw material triglyceride can be run continuously about 30 to 200 days. Even if it allows fats and oils containing obtained triglyceride to stand at a room temperature for a long period of time, there are few rises of acid value, and preservation stability is good.

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[Translation done.]

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damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.

2.\*\*\*\* shows the word which can not be translated.

3. In the drawings, any words are not translated.

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## EXAMPLE

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[Example] Hereafter, an example explains this invention still more concretely. However, this invention is not limited to these examples. That it is with "%" shows peak area % which analyzed fatty acid composition by the gas chromatograph.

Example 1 ceramic-carrier SM-10 the [NGK Insulators, Ltd. make] -- Rhizopus Delmer's (Rhizopus delemar) lipase (the Tanabe Seiyaku Co., Ltd. make.) From the upper bed of a column to the BORAJI oil after fixing "TARIPAZE" 5,000 unit / carrier g8g and stuffing the column (1.5-cm [ in diameter ], 6.2-cm [ in length ], and capacity 10.95cm<sup>3</sup>) of a cylindrical shape It is wheat germ oil (Eisai Co., Ltd. make, "IMIKKUSU") 0.2 % of the weight] as 22.2% of gamma-linolenic acid in triglyceride, [moisture content content of 200 ppm, and vitamin E. 1:2 (weight ratio) mixture of the caprylic acid (moisture content content of 200 ppm) which is medium chain fatty acid of the carbon number 8 [raw-material triglyceride: The ester interchange successive reaction was performed at 30 \*\*, teaching caprylic acid =1:2(weight ratio)] by linear velocity 4 ml/hr, and space velocity 0.589 / hr. Passage liquid 3g preparative isolation of the obtained reaction mixture was done the one-day back of a reaction start, and 90 days afterward, it added 1N-sodium hydroxide solution, neutralized, removed the water layer (lower layer) after neglect, carried out hexane extraction of the triglyceride layer (upper layer), removed this hexane, and obtained the glyceride fraction. The ODS column (AM120 S-50, product made by YMC) analyzed this glyceride fraction, and the triglyceride fraction was computed with 0.79 g. Methyl esterification of the obtained triglyceride fraction was carried out with the conventional method, capillary chromatography analyzed the fatty acid composition in triglyceride, and gamma-linolenic acid became 29.4%. [Since the triglyceride 0.79g (29.4% of gamma-linolenic acid) was obtained from 1g of raw material triglyceride (22.2% of gamma-linolenic acid), the recovery rate of triglyceride was 96-mol %.] These fats and oils 1g were put into the seal test tube, the retention test was done for one month at the room temperature, the acid value before preservation and after preservation was measured by the standard fats-and-oils assay method, the rise of acid value was measured, and it evaluated as follows.

O ... [ --- As for more than 10mgKOH / fats and oils g, the result one day after a reaction start is shown in Table 1, and the result of 90 days after is shown in Table 2. ] 1mgKOH / less than fats-and-oils g O ... 1 - 5mgKOH / less than fats-and-oils g \*\* ... 5 - 10mgKOH / less than fats-and-oils g x

[0023] In example 2 Example 1, except having used the BORAJI oil which does not contain a wheat germ oil (the Eisai Co., Ltd. make, "IMIKKUSU"), it reacted similarly, and analyzed similarly and content triglyceride was obtained for gamma-linolenic acid at recovery rate % of 94 mol to the raw material BORAJI oil 29.0%.

The retention test was done similarly. A result is shown in Tables 1 and 2.

[0024]In example 3 Example 1, instead of caprylic acid, capric acid (moisture content of 200 ppm) was carried out to \*\*\* for the said weight, and the appearance, and was evaluated similarly. A result is shown in Tables 1 and 2.

[0025]In example 4 Example 1, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0026]In example 5 Example 2, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0027]In example 6 Example 3, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0028]In comparative example 1 Example 1, used a 10 ppm thing for 10 ppm, the moisture content of caprylic acid was made to react to them like Example 1, the moisture content of the BORAJI oil was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0029]In comparative example 2 Example 1, used a 1000 ppm thing for 1000 ppm, the moisture content of caprylic acid was made to react to them like Example 1, the moisture content of the BORAJI oil was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.

[0030]

[Table 1]

	Triglyceride Retention test GLA(%)	DGLA(%)	TORIGURISEDO raw material	After a reaction	Raw material	After a reaction
Recovery rate (mol %)	example 1	22.2	29.4	---	96	O
	example 2	22.2	29.0	---	94	O
	example 3	22.2	29.0	---	96	O
	example 4	---	16.0	25.3	95	O
	example 5	---	16.0	25.0	95	O
	example 6	---	16.0	25.0	95	O
	comparative example 1	22.0	28.5	---	98	O
	comparative example 2	22.0	28.0	---	90	O

[Table 2]

	Triglyceride Retention test GLA(%)	DGLA(%)	TORIGURISEDO raw material	After a reaction	Raw material	After a reaction
Recovery rate (mol %)	example 1	22.2	29.2	---	95	O
	Example 2	22.2	29.0	---	94	O
	Example 3	22.2	29.0	---	96	O
	Example 4	---	16.0	25.3	95	O
	Example 5	---	16.0	25.0	95	O
	Example 6	---	16.0	25.0	95	O
	comparative example 1	22.0	22.2	---	100	O
	comparative example 2	22.0	23.0	---	75	O

[Translation done.]